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<b>(21) International Application Number:</b> PCT/EP98/02998  <b>(22) International Filing Date:</b> 13 May 1998 (13.05.98)  <b>(30) Priority Data:</b> 08/855,311      13 May 1997 (13.05.97)      US  <b>(71) Applicant (for all designated States except US):</b> SOCIETE DE CONSEILS DE RECHERCHES ET D'APPLICATIONS SCIENTIFIQUES S.A. (S.C.R.A.S.) [FR/FR]: 51-53, rue du Docteur Blanche, F-Paris (FR).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> CAWTHORNE, Michael, Anthony [GB/GB]; University of Buckingham, Clore Lab- oratory, Hunter Street, Buckingham, Bucks MK18 1EG (GB). LIU, Yong-Ling [GB/GB]: Clore House, Hunter Street, Buckingham, Bucks MK18 1EG (GB). SENNITT, MATTHEW, V. [GB/GB]: Clore House, Hunter Street, Buckingham, Bucks MK18 1EG (GB).  <b>(74) Agent:</b> LUNT, Mark, George, Francis; Dibb Lupton Alsop, Fountain Precinct, Balm Green, Sheffield S1 1RZ (GB).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), Eurasian patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the          claims and to be republished in the event of the receipt of          amendments.</i>
<b>(54) Title:</b> METHOD AND COMPOSITIONS FOR TREATING HYPERLIPIDEMIA AND OTHER CONDITIONS		
<b>(57) Abstract</b>  The present invention relates to a method of treating hyperlipidemia and to reducing triacylglycerols, glycerol and cholesterol in a patient. The method includes the step of administering a therapeutically effective amount of a type-5 selective somatostatin agonist to said patient. A pharmaceutical composition comprises said agonist and such product is used in the preparation of the composition for use in treating hyperlipidemia or reducing triacylglycerols, glycerol and cholesterol in a patient's body.		

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METHOD AND COMPOSITIONS FOR TREATING HYPERLIPIDEMIA AND  
OTHER CONDITIONS

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This invention relates to a method and to compositions useful in the treatment of hyperlipidemia and other conditions, for example high levels of triacylglycerols, glycerol or cholesterol in a patient.

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BACKGROUND OF THE INVENTION

There are substantial epidemiologic, clinical, genetic and experimental evidence that suggested a primary role of plasma lipids and lipoproteins in  
15 atherogenesis (Adult Treatment Panel II, Circulation 89:1333-1445 (1994); Havel, R.J., Clin. Exp. Hypertens. 11:887-900 (1989)). Atherogenesis is the process by which lipids accumulate in the intimal lining of arteries leading to the formation of plaques and hardening of the  
20 vessel wall or atherosclerosis. Although the exact mechanism leading to atherogenesis is still not well understood, abnormalities of lipid and lipoprotein metabolism, coagulation, hyperinsulinism and glycation all seem to contribute significantly to the process  
25 (Bierman, E.L., Arterio. Throm. 12:647-656 (1992)). Hyperlipidemia's characteristics of raised plasma concentrations of triglyceride, raised low density lipoprotein (LDL) cholesterol concentrations, and low concentrations of high density lipoprotein (HDL)  
30 cholesterol are known independent risk factors for atherosclerosis and its clinical sequelae, ischemic heart disease or coronary heart disease (Harrison's Principles

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of Internal Medicine, Eds. Braunwald, E., et al., 11th Edition, McGraw-Hill, 1016-1024 (1988); Reaven, GM, et al., N. Engl. J. Med. 334:374-381 (1996); and Hamsten, A., et al., N. Engl. J. Med. 313:1557-1563 (1985)).

- 5 Hyperlipidemia in clinical practice, defined by the upper 10 percent of the distribution of plasma lipid levels in a population, i.e., serum cholesterol of 205 mg/dl or higher, serum triglycerides of 200 mg/dl, is usually recommended for treatment (Havel, R.J., et al., N. Engl. J. Med. 332:1491-1498 (1995)). Routine measurements of concentrations of cholesterol and triacylglycerides in the plasma have become widespread in clinical practice which permits the identification of patients with asymptomatic hyperlipidemia. Guidelines are available for diagnosis and monitoring responses to therapy. See Workshop Treatment of Hyperlipidemia, 1996-2 (Lakesmedelsverket, Uppsala, Sweden 1996). Lowering plasma lipid concentrations reduces the amount of atherogenic plaques on the intima of blood vessels (Pathologic Basis of Disease, Eds. S.L. Robbins, et al., 3rd Edition, W.B. Saunders 506-518 (1984); Levine, G.N., et al., N. Engl. J. Med. 332:512-521 (1995)).

- A number of disorders are associated with hyperlipidemia, such as uncontrolled diabetes mellitus (insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus) (Bianchi, R., et al., Diab. Nutr. Metabl. 7:43-51 (1994); Welborn, T.A., Aust. NZ J. Med. 24:61-64 (1994)), hypothyroidism, uremia, nephrotic syndrome, acromegaly, obstructive liver disease, dysproteinemia (multiple myeloma, lupus erythematosus)

(Harrison's Principles of Internal Medicine, Ed. Braunwald, E., et al., 11th Edition, McGraw-Hill 1016-1024 (1988)). A number of drugs also produce hyperlipidemia, such as oral contraceptives, estrogens, glucocorticoids and antihypertensives. Dietary factors such as increased caloric intake (recent weight gain), consumption of foods high in saturated fats and cholesterol and alcohol intake contribute to the development of hyperlipidemia. Aside from these, primary hyperlipidemia include a family of genetic disorders associated with family histories of hyperlipidemia or xanthomas and pancreatitis.

The administration of somatostatin has been shown to reduce plasma triglyceride concentrations in alloxan diabetic dogs (Martin, C., et al., Life Sci. 35:2627-2633 (1984)), normal humans (Moller, N., et al., Clin. Sci., 75:345-350 (1988); Fukushima, H., et al., Endocrinol. Japan., 32:241-248 (1985)) and acromegalics (Cohen, R., et al., Horm. Metab. Res., 24:397-400 (1992); James, R.A., et al., Diabet. Med. 8:517 (1991)). Five distinct somatostatin receptor subtypes have been isolated. While the somatostatin type-5 receptor has been found in various areas of the brain, it has not been found in the major tissues associated with lipid metabolism, such as the liver, pancreas, and muscle. See, Bruno, et al., Endocrinology 133:2561 (1993). The present invention relates to the discovery that the somatostatin type-5 receptor is responsible for this reduction of plasma lipids.

SUMMARY OF THE INVENTION

The present invention relates to a method of treating hyperlipidemia in a patient (e.g., a mammal such as a human). The method includes the step of  
5 administering a therapeutically effective amount of a somatostatin type-5 receptor (SSTR-5) agonist (e.g., a somatostatin type-5 selective agonist) to said patient. The present invention also relates to a method of lowering the amount of cholesterol (e.g., total  
10 cholesterol or LDL cholesterol), triacylglycerols (e.g., triglycerides), or glycerol in a patient. The method includes the step of administering a therapeutically effective amount of a somatostatin type-5 receptor (SSTR-5) agonist to said patient (e.g., a somatostatin type-5  
15 receptor selective agonist). The somatostatin agonist may be administered parenterally, e.g., administered intravenously, subcutaneously, or by implantation of a sustained release formulation. In one embodiment, the patient is suffering from hyperlipidemia (e.g.,  
20 abnormally high levels of cholesterol, triacylglycerols, or glycerol) and/or is a diabetic (i.e., type-I or type-II diabetic).

The invention also provides a pharmaceutical composition comprising a therapeutically effective amount  
25 of a somatostatin type-5 receptor, optionally selective, agonist. The invention also provides the use of such agonist in the preparation of such composition for the treatment of hyperlipidemia and/or reduction in levels of triacylglycerols, glycerol or cholesterol in a human or  
30 mammalian animal.

Definitions of "somatostatin type-5 receptor agonist" and "somatostatin type-5 receptor selective agonist" will be given below. A therapeutically effective amount depends upon the condition being treated, the route of administration chosen, and the specific activity of the compound used and ultimately will be decided by the attending physician or veterinarian (e.g., between 5 :g/day and 5 mg/day). In one embodiment, the somatostatin agonist is administered to the patient until the patient's lipid levels (e.g., glycerol, triacylglycerols, or cholesterol) decrease. In another embodiment, the somatostatin agonist is administered for the lifetime of the patient.

The somatostatin agonist may be injected parenterally, e.g., intravenously, into the bloodstream of the subject being treated. However, it will be readily appreciated by those skilled in the art that the route, such as intravenous, subcutaneous, intramuscular, intraperitoneal, enterally, transdermally, transmucously, sustained released polymer compositions (e.g., a lactic acid polymer or lactic acid and glycolic acid copolymer microparticle or implant), perfusion, nasal, oral, etc., will vary with the condition being treated and the activity and bioavailability of the somatostatin agonist being used.

While it is possible for the somatostatin agonist to be administered as the pure or substantially pure compound, it may also be presented as a pharmaceutical formulation or preparation. The formulations to be used

in the present invention, for both humans and animals, comprise any of the somatostatin agonists to be described below, together with one or more pharmaceutically acceptable carriers thereof, and optionally other  
5 therapeutic ingredients.

The carrier must be "acceptable" in the sense of being compatible with the active ingredient(s) of the formulation (e.g., capable of stabilizing peptides) and not deleterious to the subject to be treated. Desirably,  
10 the formulation should not include oxidizing agents or other substances with which peptides are known to be incompatible. For example, somatostatin agonists in the cyclized form (e.g., internal cysteine disulfide bond) can be oxidized; thus, the presence of reducing agents as  
15 excipients could lead to an opening of the cysteine disulfide bridge. On the other hand, highly oxidative conditions can lead to the formation of cysteine sulfoxide and to the oxidation of tryptophane. Consequently, it is important to carefully select the  
20 excipient. pH is another key factor, and it may be necessary to buffer the product under slightly acidic conditions (pH 5 to 6).

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the  
25 methods well known in the art of pharmacy. All methods include the step of bringing the active ingredient(s) into association with the carrier which constitutes one or more accessory ingredients.

In general, the formulations for tablets or  
30 powders are prepared by uniformly and intimately blending



the active ingredient with finely divided solid carriers, and then, if necessary, as in the case of tablets, forming the product into the desired shape and size.

Formulations suitable for parenteral (e.g., intravenous) administration, on the other hand, conveniently comprise sterile aqueous solutions of the active ingredient(s). Preferably, the solutions are isotonic with the blood of the subject to be treated. Such formulations may be conveniently prepared by dissolving solid active ingredient(s) in water to produce an aqueous solution, and rendering said solution sterile.

The formulation may be presented in unit or multi-dose containers, for example, sealed ampoules or vials.

Formulations suitable for sustained release parenteral administrations (e.g., biodegradable polymer formulations such as polyesters containing lactic or glycolic acid residues) are also well known in the art. See, e.g., U.S. Patent Nos. 3,773,919 and 4,767,628 and PCT Publication No. WO 94/15587.

The somatostatin or somatostatin agonist may also be administered with another compound capable of lowering blood levels of triglycerides, cholesterol, or glycerol, such as fibrates (e.g., bezafibrate, gemfibrozil, and clofibrate), HMG-COA reductase inhibitors (e.g., pravastatin, simvastatin, and fluvastatin, Atorvastatin, and Lovastatin), bile acid binding resins (e.g., cholestyramine and colestipol), nicotinic acid compounds (e.g., nicotinic acid and niceritrol), and fish oils. See Workshop Treatment of Hyperlipidemia 1996-2 (Lakemedelsverket, Uppsala, Sweden, 1996).

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments and from the claims.

5                   DETAILED DESCRIPTION OF THE INVENTION

It is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely  
10 illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art  
15 to which this invention belongs. Also, all publications, patent applications, patents, and other references mentioned herein are incorporated by reference.

Somatostatin and its Agonists

20           Somatostatin (somatotropin release inhibiting factor or SRIF) has both a 14 amino acid isoform (somatostatin-14) and a 28 amino acid isoform (somatostatin-28). See Wilson, J. & Foster, D., *Williams Textbook of Endocrinology*, p. 510 (7th ed., 1985). The  
25 compound is an inhibitor of secretion of the growth hormone and was originally isolated from the hypothalamus. Brazeau, et al., *Science* 179:77 (1973). Native somatostatin has a very short duration of effect *in vivo* since it is rapidly inactivated by endo- and  
30 exopeptidase. Many novel analogs (e.g., peptide and non-

peptide compounds) have been prepared in order to enhance the duration of effect, biological activity, and selectivity (e.g., for the particular somatostatin receptor) of this hormone. Such analogs of somatostatin will be called "somatostatin agonists" herein.

Various somatostatin receptors (SSTRs) have been isolated, e.g., SSTR-1, SSTR-2, SSTR-3, SSTR-4, and SSTR-5. Thus, the somatostatin agonist may be a SSTR-1 agonist, SSTR-2 agonist, SSTR-3 agonist, SSTR-4 agonist or a SSTR-5 agonist. What is meant by a somatostatin type-5 receptor agonist (i.e., SSTR-5 agonist) is a compound which (1) has a high binding affinity (e.g.,  $K_i$  of less than 5 nM or preferably less than 2 nM or less than 1 nM) for SSTR-5 (e.g., as defined by the receptor binding assay described below) and (2) decreases lipid levels (e.g., cholesterol, glycerols, or triacylglycerols) in a patient (e.g., as shown by the biological assay described below). What is meant by a somatostatin type-5 receptor selective agonist is a somatostatin agonist which (1) has a higher binding affinity (i.e.,  $K_i$ ) for SSTR-5 than for either SSTR-1, SSTR-2, SSTR-3, or SSTR-4 and (2) decreases lipid levels (e.g., cholesterol, glycerols, or triacylglycerols) in a patient (e.g., as shown by the biological assay described below). In one embodiment, the SSTR-5 selective agonist has a  $K_i$  for SSTR-5 that is at least 2 times (e.g., at least 5 times or at least 10 times) less than its  $K_i$  for the SSTR-2 receptor (e.g., as defined by the receptor binding assay described below). In one embodiment, the

somatostatin type-5 receptor selective agonist is also a SSTR-5 agonist.

Examples of somatostatin agonists are those covered by formulae or those specifically recited in the publications set forth below, all of which are hereby incorporated by reference.

EP Application No. P5 164 EU (Inventor: G. Keri);  
Van Binst, G. et al. Peptide Research 5:8 (1992);  
Horvath, A. et al. Abstract, "Conformations of  
10 Somatostatin Analogs Having Antitumor Activity", 22nd  
European peptide Symposium, September 13-19, 1992,  
Interlaken, Switzerland;

PCT Application No. WO 91/09056 (1991);  
EP Application No. 0 363 589 A2 (1990);  
15 U.S. Patent No. 4,904,642 (1990);  
U.S. Patent No. 4,871,717 (1989);  
U.S. Patent No. 4,853,371 (1989);  
U.S. Patent No. 4,725,577 (1988);  
U.S. Patent No. 4,684,620 (1987);  
20 U.S. Patent No. 4,650,787 (1987);  
U.S. Patent No. 4,603,120 (1986);  
U.S. Patent No. 4,585,755 (1986);  
EP Application No. 0 203 031 A2 (1986);  
U.S. Patent No. 4,522,813 (1985);  
25 U.S. Patent No. 4,486,415 (1984);  
U.S. Patent No. 4,485,101 (1984);  
U.S. Patent No. 4,435,385 (1984);  
U.S. Patent No. 4,395,403 (1983);  
U.S. Patent No. 4,369,179 (1983);  
30 U.S. Patent No. 4,360,516 (1982);

- U.S. Patent No. 4,358,439 (1982);  
U.S. Patent No. 4,328,214 (1982);  
U.S. Patent No. 4,316,890 (1982);  
U.S. Patent No. 4,310,518 (1982);  
5 U.S. Patent No. 4,291,022 (1981);  
U.S. Patent No. 4,238,481 (1980);  
U.S. Patent No. 4,235,886 (1980);  
U.S. Patent No. 4,224,199 (1980);  
U.S. Patent No. 4,211,693 (1980);  
10 U.S. Patent No. 4,190,648 (1980);  
U.S. Patent No. 4,146,612 (1979);  
U.S. Patent No. 4,133,782 (1979);  
U.S. Patent No. 5,506,339 (1996);  
U.S. Patent No. 4,261,885 (1981);  
15 U.S. Patent No. 4,728,638 (1988);  
U.S. Patent No. 4,282,143 (1981);  
U.S. Patent No. 4,215,039 (1980);  
U.S. Patent No. 4,209,426 (1980);  
U.S. Patent No. 4,190,575 (1980);  
20 EP Patent No. 0 389 180 (1990);  
EP Application No. 0 505 680 (1982);  
EP Application No. 0 083 305 (1982);  
EP Application No. 0 030 920 (1980);  
PCT Application No. WO 88/05052 (1988);  
25 PCT Application No. WO 90/12811 (1990);  
PCT Application No. WO 97/01579 (1997);  
PCT Application No. WO 91/18016 (1991);  
U.K. Application No. GB 2,095,261 (1981); and  
French Application No. FR 2,522,655 (1983).

Examples of SSTR-5 selective somatostatin agonists include, but are not limited to, the following somatostatin analogs which are disclosed in the above-cited references:

- 5        H-Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> (BIM-23268);  
          H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub> (BIM-23052);  
          H-Cys-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys-NH<sub>2</sub> (BIM-23284);  
          H-Cys-Phe-Tyr-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> (BIM-23295);  
          H-Cys-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> (BIM-23313);

10        HO(CH<sub>2</sub>)<sub>2</sub>-N        N-(CH<sub>2</sub>)-CO-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>  
          (BIM-23272); and

         HO(CH<sub>2</sub>)<sub>2</sub>-N        N-(CH<sub>2</sub>)<sub>2</sub>-SO<sub>2</sub>-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>

Note that for all somatostatin agonists described  
 15    herein, each amino acid residue represents the structure  
      of -NH-C(R)H-CO-, in which R is the side chain (e.g., CH<sub>3</sub>  
      for Ala). Lines between amino acid residues represent  
      peptide bonds which join the amino acids. Also, where  
      the amino acid residue is optically active, it is the L-  
 20    form configuration that is intended unless D-form is  
      expressly designated. A disulfide bond (e.g., a  
      disulfide bridge) exists between the two free thiols of  
      the Cys residues; however, it is not shown.

## 25    Synthesis of somatostatin agonists

The methods for synthesizing somatostatin agonists is well documented and are within the ability of a person of ordinary skill in the art.

Synthesis of short amino acid sequences is well established in the peptide art. For example, synthesis of H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>, described above, can be achieved by following the protocol set forth in Example I of European Patent Application 0 395 417 A1. The synthesis of somatostatin agonists with a substituted N-terminus can be achieved, for example, by following the protocol set forth in WO 88/02756, European Patent Application No. 0 329 295, and PCT Publication No. WO 94/04752.

#### Somatostatin Receptor Binding Assays

The human SSTR-1, SSTR-2, SSTR-3, SSTR-4, and SSTR-5 cDNA clones have been described (SSTR-1 and SSTR-2 in Yamada, Y., et al., Proc. Natl. Acad. Sci. USA., 89:251-255 (1992); SSTR-3 in Yamada, et al., Mol. Endocrinol. 6:2136-2142 (1993); and SSTR-4 and SSTR-5 in Yamada, et al., Biochem. Biophys. Res. Commun. 195:844-852 (1993)) and are also available from American Type Culture Collection (ATCC, Rockville, MD) (ATCC Nos. 79044 (SSTR-1), 79046 (SSTR-2), and 79048 (SSTR-3)). Based on the restriction endonuclease maps, the entire coding region of each SSTR cDNA may be excised by suitable restriction endonuclease digestion (Maniatis, T., et al., *Molecular Cloning - A Laboratory Manual*, CSHL, 1982). Restriction endonucleases are available from New England Biolabs (Beverly, MA). This cDNA fragment was inserted into the mammalian expression vector, pCMV (Russell, D., et al., J. Biol. Chem., 264:8222-8229 (1989)), using standard molecular biology techniques (see e.g., Maniatis, T., et al., *Molecular Cloning, -A Laboratory*

Manual, Cold Spring Harbor Laboratory, 1982) to produce the expression plasmid, pCMV-human SSTR-1 through pCMV-human SSTR-5. Other mammalian expression vectors include pcDNA1/Amp (Invitrogen, Sandlesy, CA). The expression  
5 plasmids were introduced into the suitable bacterial host, E. Coli HB101 (Stratagene, La Jolla, CA) and plasmid DNAs, for transfection, were prepared on Cesium Chloride gradients.

CHO-K1 (ovary, Chinese hamster) cells were  
10 obtained from ATCC (ATCC No. CCL 61). The cells were grown and maintained in Ham's F12 media (Gibco BRL, Grand Island, NY) supplemented with 10% fetal bovine serum under standard tissue culture conditions. For transfection, the cells were seeded at a density  $1 \times 10^6$ /60-cm plate (Baxter Scientific Products, McGraw Park,  
15 IL.). DNA mediated transfection was carried out using the calcium phosphate co-precipitation method (Ausubel, F.M., et al., Current Protocols in Molecular Biology, John Wiley & Sons, 1987). The plasmid pRSV-neo (ATCC;  
20 ATCC No. 37198) was included as a selectable marker at 1/10 the concentration of the expression plasmid. CHO-K1 clonal cell lines that have stably inherited the transfected DNA were selected for growth in Ham's F12 media containing 10% fetal bovine serum and 0.5mg/ml of  
25 G418 (Sigma). The cells were ring-cloned and expanded in the same media for analysis.

Expression of the human SSTR-1 through SSTR-5 receptors in the CHO-K1 cells were detected by Northern blot analysis of total RNA prepared from the cells  
30 (Sambrook, J.E., et al., Molecular Cloning - A Laboratory



Manual, Ed. 2., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989) and by receptor binding using [ $^{125}$ I-Tyr $^{11}$ ]somatostatin-14 as a ligand. Transfected cell lines expressing the human SSTR receptors were clonally expanded in culture and used in the following SSTR binding protocol.

Crude membranes were prepared by homogenization of the transfected cells in 20 ml of ice-cold 50 mM Tris-HCl with a POLYTRON homogenizer (setting 6, 15 sec). Buffer was added to obtain a final volume of 40 ml, and the homogenate was centrifuged in a Sorval SS-34 rotor at 39,000 g for 10 min at 0-4°C. The resulting supernatant was decanted and discarded. The pellet was rehomogenized in ice-cold buffer, diluted, and centrifuged as before. The final pellet was resuspended in the 10 mM Tris HCl and held on ice for the receptor binding assay.

Aliquots of the membrane preparation were incubated for 30 min at 30°C with 0.05 nM [ $^{125}$ I-Tyr $^{11}$ ]somatostatin-14 (2000 Ci/mmol; Amersham Corp., Arlington Heights, IL) in 50 mM HEPES (pH 7.4) containing a test somatostatin agonist of various concentrations (e.g.,  $10^{-11}$  to  $10^{-6}$ ), 10 mg/ml bovine serum albumin (fraction V) (Sigma Chemical Co., St. Louis, MO), MgCl $_2$  (5 mM), Trasylol (200 KIU ml), bacitracin (0.02 mg/ml), and phenylmethanesulphonyl fluoride (0.02 mg/ml). The final assay volume was 0.3 ml. The incubations were terminated by rapid filtration through GF/C filters (pre-soaked in 0.3% polyethylenimine for 30 min) using a Brandel filtration manifold. Each tube and filter were then

washed three times with 5 ml aliquots of ice-cold buffer.

Specific binding was defined as the total [ $^{125}$ I-Tyr $^{11}$ ]SRIF-14 bound minus that bound in the presence of 1000 nM. The  $K_i$  values for the tested somatostatin

agonists were calculated by using the following formula:

$K_i = IC_{50} / [1 + (LC/LEC)]$  where  $IC_{50}$  is the concentration of test somatostatin agonist required to inhibit 50 percent of the specific binding of the radioligand [ $^{125}$ I-Tyr $^{11}$ ]somatostatin-14, LC is the concentration of the

radioligand (0.05 nM), and LEC is the equilibrium dissociation constant of the radioligand (0.16 nM). The  $K_i$  values (nM) for the tested somatostatin agonists are shown in Table I.

TABLE I

	hSSTR-1	hSSTR-2	hSSTR-3	hSSTR-4	hSSTR-5
Somatostatin-14	2.26	0.23	1.2	1.8	1.41
Somatostatin-28	2.38	0.30	1.3	7.93	0.4
BIM-23268	1227	15.06	545	3551	0.42
BIM-23052	97.6	11.96	5.6	127	1.22
BIM-23272	47.7	3.23	10.9	753	1.01
BIM-23284	27.9	19.3	35.6	58.6	0.85
BIM-23295	86.9	6.19	9.7	3.4	0.34
BIM-23313	151	4.78	25.5	55.3	0.30

#### Reduction of Glycerol and Triglycerides

The obese (fa/fa) Zucker and its derivative in the Zucker diabetic fatty (ZDF/Drt-fa) are excellent models of diabetes-induced dyslipidemia (Shafrir, E.,

Diabetes/Metabolism Rev. 8:179-208 (1992); Peterson,

R.G., et al., ILAR News 32:16-19 (1990)). The animals develop progressive hypertriglyceridemia and hypercholesterolemia.

The effect of chronic treatment with BIM-23268 on plasma lipids was examined in an obese animal model, the fatty (fa/fa) Zucker rats (Bray, G., Federation Proceedings 36:q48-153 (1977)) (purchased from Harlan-Olac, Bicester, Oxon, U.K.). Eleven male fatty Zucker rats weighing about 450 grams were randomly divided into 2 groups and their initial body weights recorded. The animals were housed in pairs in a normal 12 hour light/dark cycle at 20 " 21C and fed a standard laboratory rat diet (Beekay rat and mouse diet, Bantin & Kingman, Hull, Humberside, U.K.) overnight *ad libitum*.

For the group assigned to receive drug treatment, the rats received BIM-23268C at 3 mg/kg, by subcutaneous injection twice a day at 10:00 a.m. and 5:00 p.m. The other group was treated with a subcutaneous injection of 0.1 ml/100 g of saline twice a day at 10:00 a.m. and 5:00 p.m. The animals were subjected to the BIM-23268 or saline treatment for a total of six days.

On the last day of treatment (day 6), food was removed at 5:00 p.m. and the rats fasted overnight. At 9:00 a.m. the next day, the animals were subjected to a glucose challenge, given as a 0.8 gram/kg of glucose orally. Periodic 400 ul of blood samples were taken from the tail vein (Peterson, R.G., ILAR News 32:16-19 (1990)) at 60 min. and 30 min. before, and at 30, 60, 90, and 120 min. after the administration of the glucose challenge (0.8 gram/kg orally). Aprotinin (Traysylol, Bayer UK,

Hayward's Heath, W. Sussex, U.K.) and heparin (Sigma Chemical Co., Poole, Dorset, U.K.) were added to the blood samples to a final concentration of 400 KIU/ml and 100 units/ml, respectively. Plasma fractions were prepared from these samples by centrifugation at 400 x G in a microfuge, for the estimation of triglycerides and glycerol. Samples were then stored at -80°C until assayed.

Plasma glycerol and triglycerides were determined using the Sigma Enzymatic (Tinder) calorimetric assay kit (Cat #337-B, Sigma Chemical Co., Poole, Dorset, U.K.) and measuring absorbance at 540 nm in a spectrophotometer.

After 6 days of treatment with BIM-23268C at 3 mg/kg, twice a day by subcutaneous injection, both plasma glycerol and triglycerides were significantly lowered, as exemplified by the samples taken at time 30 and 60 min. before the oral glucose challenge. See Fig. 1 and Fig. 2. The administration of an oral glucose challenge had no significant effect on plasma lipids. The BIM-23268C treated group showed significantly lower plasma glycerol and triglycerides through the 2-hour test period. The results suggested that BIM-23268C, following a 6-day treatment period at the prescribed dose was effective in reducing hypertriglyceridemia.

#### Measure of Efficacy in Patient

The effect of the somatostatin agonist will be assessed for a reduction in total cholesterol, total triglycerides, and total LDL cholesterol (e.g., as described in Dubrey, S.W., et al., Diabetes 43:831-835

(1994). The long term effect of the drug is examined by the change in coronary artery disease (Reviewed in Donahue, The Endocrinologist, 4:112-116 (1994)).

5

#### OTHER EMBODIMENTS

The foregoing description has been limited to specific embodiments of this invention. It will be apparent, however, that variations and modifications may be made to the invention, with the attainment of some or  
10 all of the advantages of the invention. Such embodiments are also within the scope of the following claims.

CLAIMS

1. A method of treating hyperlipidemia in a patient, said method comprising administering a  
5 therapeutically effective amount of a somatostatin type-5 receptor agonist to said patient.

2. A method of claim 1, wherein said somatostatin type-5 receptor agonist has a  $K_i$  of less than 2 nM for the somatostatin type-5 receptor.

10 3. A method of treating hyperlipidemia in a patient, said method comprising administering a therapeutically effective amount of a somatostatin type-5 receptor selective agonist to said patient.

4. A method of claim 3, wherein said  
15 somatostatin type-5 receptor selective agonist has a  $K_i$  for the type-5 somatostatin receptor that is at least 10 times less than its  $K_i$  for the somatostatin type-2 receptor.

5. A method of claim 1, said method comprising  
20 administering a therapeutically effective amount of H-Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub>, where a disulfide bond exists between the free thiols of the two Cys residues, or H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>.

6. A method of lowering the amount of  
25 triacylglycerols, glycerol, or cholesterol in the blood of a patient, said method comprising administering a therapeutically effective amount of a somatostatin type-5 receptor agonist to said patient.

7. A method of lowering the amount of  
30 triacylglycerols, glycerol, or cholesterol in the blood

of a patient, said method comprising administering a therapeutically effective amount of a somatostatin type-5 receptor selective agonist to said patient.

8. A method of claim 6, wherein said method  
5 comprises lowering the amount of triacylglycerols in said patient.

9. A method of claim 8, wherein said somatostatin type-5 receptor agonist has a  $K_i$  of less than 2 nM for the somatostatin type-5 receptor.

10 10. A method of claim 7, wherein said method comprises lowering the amount of triacylglycerols in said patient.

11. A method of claim 10, wherein said somatostatin type-5 receptor selective agonist has a  $K_i$   
15 for the type-5 somatostatin receptor that is at least 10 times less than its  $K_i$  for the somatostatin type-2 receptor.

12. A method of claim 8, said method comprising  
administering a therapeutically effective amount of H-Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub>, where a disulfide  
20 bond exists between the free thiols of the two Cys residues, or H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>.

13. A method of claim 6, wherein said method  
comprises lowering the amount of glycerol in said  
25 patient.

14. A method of claim 13, wherein said somatostatin type-5 receptor agonist has a  $K_i$  of less than 2 nM for the somatostatin type-5 receptor.

15. A method of claim 7, wherein said method comprises lowering the amount of glycerol in said patient.

16. A method of claim 15, wherein said  
5 somatostatin type-5 receptor selective agonist has a  $K_i$  for the type-5 somatostatin receptor that is at least 10 times less than its  $K_i$  for the somatostatin type-2 receptor.

17. A method of claim 13, said method comprising  
10 administering a therapeutically effective amount of H-Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub>, where a disulfide bond exists between the free thiols of the two Cys residues, or H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>.

18. A method of claim 6, wherein said method  
15 comprises lowering the amount of cholesterol in said patient.

19. A method of claim 18, wherein said somatostatin type-5 receptor agonist has a  $K_i$  of less than 2 nM for the somatostatin type-5 receptor.

20. A method of claim 7, wherein said method  
20 comprises lowering the amount of total cholesterol or LDL cholesterol in said patient.

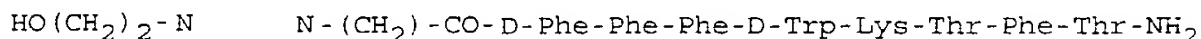
21. A method of claim 20, wherein said somatostatin type-5 receptor selective agonist has a  $K_i$   
25 for the type-5 somatostatin receptor that is at least 10 times less than its  $K_i$  for the somatostatin type-2 receptor.

22. A method of claim 18, said method comprising administering a therapeutically effective amount of H-  
30 Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub>, where a disulfide

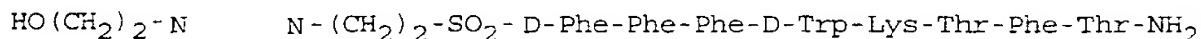


bond exists between the free thiols of the two Cys residues, or H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>.

23. A method according to claim 1 wherein the somatostatin type-5 receptor agonist is
- 5 H-Cys-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys-NH<sub>2</sub> ,  
 H-Cys-Phe-Tyr-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> ,  
 H-Cys-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> ,

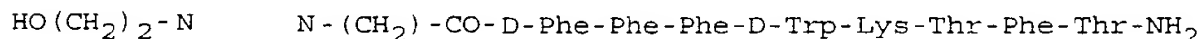


or

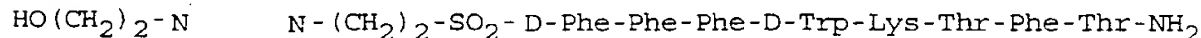


10

24. A method according to claim 8 wherein the somatostatin type-5 receptor agonist is
- H-Cys-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys-NH<sub>2</sub> ,
- 15 H-Cys-Phe-Tyr-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> ,  
 H-Cys-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> ,



or



20

25. A method according to claim 13 wherein the somatostatin type-5 receptor agonist is
- H-Cys-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys-NH<sub>2</sub> ,
- H-Cys-Phe-Tyr-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> ,

H-Cys-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> ,

HO(CH<sub>2</sub>)<sub>2</sub>-N — N-(CH<sub>2</sub>)<sub>2</sub>-CO-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>

or

HO(CH<sub>2</sub>)<sub>2</sub>-N — N-(CH<sub>2</sub>)<sub>2</sub>-SO<sub>2</sub>-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>

5 .

26. A method according to claim 18 wherein the somatostatin type-5 receptor agonist is

H-Cys-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys-NH<sub>2</sub> ,

10 H-Cys-Phe-Tyr-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> ,

H-Cys-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> ,

HO(CH<sub>2</sub>)<sub>2</sub>-N — N-(CH<sub>2</sub>)<sub>2</sub>-CO-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>

or

HO(CH<sub>2</sub>)<sub>2</sub>-N — N-(CH<sub>2</sub>)<sub>2</sub>-SO<sub>2</sub>-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>

15 .

27. A pharmaceutical composition comprising a therapeutically effective amount of a somatostatin type-5 receptor, optionally selective, agonist.

20 28. A pharmaceutical composition as claimed in claim 27, said agonist having the features identified in any one of claims 2, 4, 5 and 23 to 26.

29. Use of a somatostatin type-5 receptor, optionally selective, agonist in the formulation of a  
25 pharmaceutical composition for use in treating hyperlipidemia, or reducing the amount of

tracylglycerols, glycerol, or cholesterol in a human or mammalian animal.

30. Use of a somatostatin agonist according to claim 29, wherein said somatostatin agonist has the  
5 relevant features identified in any one of claims 2, 4, 5 and 23 to 26.

31. A pharmaceutical composition substantially as hereinbefore described with reference to the Examples.

# INTERNATIONAL SEARCH REPORT

Int. Patent Application No.

PCT/EP 98/02998

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K38/31

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 35950 A (UNIVERSITY OF BUCKINGHAM) 14 November 1996 see page 5, line 1 - line 3; claims 10-15 see page 8, line 24 - page 9, line 10 ---	27, 28, 31
X	WO 97 11962 A (BIOMEASURE INCORPORATED ET AL.) 3 April 1997 see page 6, line 12 - line 23; claim 21 see page 12, line 22 - page 13, line 35; table 1 --- -/--	27, 28, 31

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

22 September 1998

Date of mailing of the international search report

07/10/1998

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 98/02998

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DAVENPORT M ET AL: "SOMATOSTATIN TYPE 5 RECEPTORS MEDiate THE INHIBITORY ACTION OF SOMATOSTATIN ON INSULIN SECRETION IN ZUCKER FATTY RAT PERFUSED PANCREAS" DIABETOLOGIA, vol. 38, no. SUPPL. 01, 1995, page A106 XP000602793 see abstract nr. 410 ---	1-31
A	N. MOLLER ET AL.: "EFFECTS OF THE SOMATOSTATIN ANALOGUE SMS 201-995 (SANDOSTATIN) ON MOUTH-TO-CAECUM TRANSIT TIME AND ABSORPTION OF FAT AND CARBOHYDRATES IN NORMAL MAN." CLINICAL SCIENCE, vol. 75, no. 4, October 1988, pages 345-350, XP002078328 LONDON, GB cited in the application see the whole document ---	1-31
P,X	WO 98 10786 A (Y. COHEN) 19 March 1998  see page 1. line 29 - line 31; claims 1, 19, 58 see page 3. line 13 - page 4. line 13 see page 23. line 28 - page 24. line 5 -----	1-22, 27-31

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 98/02998

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 1-26  
are directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/EP 98/02998

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9635950 A	14-11-1996	US 5763200 A AU 694360 B AU 5764596 A CA 2220106 A EP 0829011 A	09-06-1998 16-07-1998 29-11-1996 14-11-1996 18-03-1998
WO 9711962 A	03-04-1997	US 5708135 A AU 6914596 A EP 0859785 A NO 981395 A	13-01-1998 17-04-1997 26-08-1998 27-03-1998
WO 9810786 A	19-03-1998	AU 4133997 A	02-04-1998







## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 38/31</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 98/51330</b> <b>(43) International Publication Date:</b> 19 November 1998 (19.11.98)
<b>(21) International Application Number:</b> PCT/EP98/02998 <b>(22) International Filing Date:</b> 13 May 1998 (13.05.98)  <b>(30) Priority Data:</b> 08/855.311 13 May 1997 (13.05.97) US  <b>(71) Applicant (for all designated States except US):</b> SOCIETE DE CONSEILS DE RECHERCHES ET D'APPLICATIONS SCIENTIFIQUES S.A. (S.C.R.A.S.) [FR/FR]; 51-53, rue du Docteur Blanche, F-Paris (FR).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> CAWTHORNE, Michael, Anthony [GB/GB]; University of Buckingham, Clore Lab- oratory, Hunter Street, Buckingham, Bucks MK18 1EG (GB). LIU, Yong-Ling [GB/GB]; Clore House, Hunter Street, Buckingham, Bucks MK18 1EG (GB). SENNITT, MATTHEW, V. [GB/GB]; Clore House, Hunter Street, Buckingham, Bucks MK18 1EG (GB).  <b>(74) Agent:</b> LUNT, Mark, George, Francis; Dibb Lupton Alsop, Fountain Precinct, Balm Green, Sheffield S1 1RZ (GB).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> METHOD AND COMPOSITIONS FOR TREATING HYPERLIPIDEMIA AND OTHER CONDITIONS		
<b>(57) Abstract</b>  The present invention relates to a method of treating hyperlipidemia and to reducing triacylglycerols, glycerol and cholesterol in a patient. The method includes the step of administering a therapeutically effective amount of a type-5 selective somatostatin agonist to said patient. A pharmaceutical composition comprises said agonist and such product is used in the preparation of the composition for use in treating hyperlipidemia or reducing triacylglycerols, glycerol and cholesterol in a patient's body.		

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METHOD AND COMPOSITIONS FOR TREATING HYPERLIPIDEMIA AND  
OTHER CONDITIONS

5

This invention relates to a method and to compositions useful in the treatment of hyperlipidemia and other conditions, for example high levels of triacylglycerols, glycerol or cholesterol in a patient.

10

BACKGROUND OF THE INVENTION

There are substantial epidemiologic, clinical, genetic and experimental evidence that suggested a primary role of plasma lipids and lipoproteins in  
15 atherogenesis (Adult Treatment Panel II, Circulation 89:1333-1445 (1994); Havel, R.J., Clin. Exp. Hypertens. 11:887-900 (1989)). Atherogenesis is the process by which lipids accumulate in the intimal lining of arteries leading to the formation of plaques and hardening of the  
20 vessel wall or atherosclerosis. Although the exact mechanism leading to atherogenesis is still not well understood, abnormalities of lipid and lipoprotein metabolism, coagulation, hyperinsulinism and glycation all seem to contribute significantly to the process  
25 (Bierman, E.L., Arterio. Throm. 12:647-656 (1992)). Hyperlipidemia's characteristics of raised plasma concentrations of triglyceride, raised low density lipoprotein (LDL) cholesterol concentrations, and low concentrations of high density lipoprotein (HDL)  
30 cholesterol are known independent risk factors for atherosclerosis and its clinical sequelae, ischemic heart disease or coronary heart disease (Harrison's Principles

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of Internal Medicine, Eds. Braunwald, E., et al., 11th Edition, McGraw-Hill, 1016-1024 (1988); Reaven, GM, et al., N. Engl. J. Med. 334:374-381 (1996); and Hamsten, A., et al., N. Engl. J. Med. 313:1557-1563 (1985)).

5 Hyperlipidemia in clinical practice, defined by the upper 10 percent of the distribution of plasma lipid levels in a population, i.e., serum cholesterol of 205 mg/dl or higher, serum triglycerides of 200 mg/dl, is usually recommended for treatment (Havel, R.J., et al., N. Engl. 10 J. Med. 332:1491-1498 (1995)). Routine measurements of concentrations of cholesterol and triacylglycerides in the plasma have become widespread in clinical practice which permits the identification of patients with asymptomatic hyperlipidemia. Guidelines are available 15 for diagnosis and monitoring responses to therapy. See Workshop Treatment of Hyperlipidemia, 1996-2 (Lakesmedelsverket, Uppsala, Sweden 1996). Lowering plasma lipid concentrations reduces the amount of atherogenic plaques on the intima of blood vessels 20 (Pathologic Basis of Disease, Eds. S.L. Robbins, et al., 3rd Edition, W.B. Saunders 506-518 (1984); Levine, G.N., et al., N. Engl. J. Med. 332:512-521 (1995)).

A number of disorders are associated with hyperlipidemia, such as uncontrolled diabetes mellitus 25 (insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus) (Bianchi, R., et al., Diab. Nutr. Metabl. 7:43-51 (1994); Welborn, T.A., Aust. NZ J. Med. 24:61-64 (1994)), hypothyroidism, uremia, nephrotic syndrome, acromegaly, obstructive liver disease, 30 dysproteinemia (multiple myeloma, lupus erythematosus)

(Harrison's Principles of Internal Medicine, Ed. Braunwald, E., et al., 11th Edition, McGraw-Hill 1016-1024 (1988)). A number of drugs also produce hyperlipidemia, such as oral contraceptives, estrogens, glucocorticoids and antihypertensives. Dietary factors such as increased caloric intake (recent weight gain), consumption of foods high in saturated fats and cholesterol and alcohol intake contribute to the development of hyperlipidemia. Aside from these, primary hyperlipidemia include a family of genetic disorders associated with family histories of hyperlipidemia or xanthomas and pancreatitis.

The administration of somatostatin has been shown to reduce plasma triglyceride concentrations in alloxan diabetic dogs (Martin, C., et al., Life Sci. 35:2627-2633 (1984)), normal humans (Moller, N., et al., Clin. Sci., 75:345-350 (1988); Fukushima, H., et al., Endocrinol. Japan., 32:241-248 (1985)) and acromegalics (Cohen, R., et al., Horm. Metab. Res., 24:397-400 (1992); James, R.A., et al., Diabet. Med. 8:517 (1991). Five distinct somatostatin receptor subtypes have been isolated. While the somatostatin type-5 receptor has been found in various areas of the brain, it has not been found in the major tissues associated with lipid metabolism, such as the liver, pancreas, and muscle. See, Bruno, et al., Endocrinology 133:2561 (1993). The present invention relates to the discovery that the somatostatin type-5 receptor is responsible for this reduction of plasma lipids.

SUMMARY OF THE INVENTION

The present invention relates to a method of treating hyperlipidemia in a patient (e.g., a mammal such as a human). The method includes the step of  
5 administering a therapeutically effective amount of a somatostatin type-5 receptor (SSTR-5) agonist (e.g., a somatostatin type-5 selective agonist) to said patient. The present invention also relates to a method of lowering the amount of cholesterol (e.g., total  
10 cholesterol or LDL cholesterol), triacylglycerols (e.g., triglycerides), or glycerol in a patient. The method includes the step of administering a therapeutically effective amount of a somatostatin type-5 receptor (SSTR-5) agonist to said patient (e.g., a somatostatin type-5  
15 receptor selective agonist). The somatostatin agonist may be administered parenterally, e.g., administered intravenously, subcutaneously, or by implantation of a sustained release formulation. In one embodiment, the patient is suffering from hyperlipidemia (e.g.,  
20 abnormally high levels of cholesterol, triacylglycerols, or glycerol) and/or is a diabetic (i.e., type-I or type-II diabetic).

The invention also provides a pharmaceutical composition comprising a therapeutically effective amount  
25 of a somatostatin type-5 receptor, optionally selective, agonist. The invention also provides the use of such agonist in the preparation of such composition for the treatment of hyperlipidemia and/or reduction in levels of triacylglycerols, glycerol or cholesterol in a human or  
30 mammalian animal.

Definitions of "somatostatin type-5 receptor agonist" and "somatostatin type-5 receptor selective agonist" will be given below. A therapeutically effective amount depends upon the condition being treated, the route of administration chosen, and the specific activity of the compound used and ultimately will be decided by the attending physician or veterinarian (e.g., between 5 :g/day and 5 mg/day). In one embodiment, the somatostatin agonist is administered to the patient until the patient's lipid levels (e.g., glycerol, triacylglycerols, or cholesterol) decrease. In another embodiment, the somatostatin agonist is administered for the lifetime of the patient.

The somatostatin agonist may be injected parenterally, e.g., intravenously, into the bloodstream of the subject being treated. However, it will be readily appreciated by those skilled in the art that the route, such as intravenous, subcutaneous, intramuscular, intraperitoneal, enterally, transdermally, transmucously, sustained released polymer compositions (e.g., a lactic acid polymer or lactic acid and glycolic acid copolymer microparticle or implant), perfusion, nasal, oral, etc., will vary with the condition being treated and the activity and bioavailability of the somatostatin agonist being used.

While it is possible for the somatostatin agonist to be administered as the pure or substantially pure compound, it may also be presented as a pharmaceutical formulation or preparation. The formulations to be used

in the present invention, for both humans and animals, comprise any of the somatostatin agonists to be described below, together with one or more pharmaceutically acceptable carriers thereof, and optionally other  
5 therapeutic ingredients.

The carrier must be "acceptable" in the sense of being compatible with the active ingredient(s) of the formulation (e.g., capable of stabilizing peptides) and not deleterious to the subject to be treated. Desirably,  
10 the formulation should not include oxidizing agents or other substances with which peptides are known to be incompatible. For example, somatostatin agonists in the cyclized form (e.g., internal cysteine disulfide bond) can be oxidized; thus, the presence of reducing agents as  
15 excipients could lead to an opening of the cysteine disulfide bridge. On the other hand, highly oxidative conditions can lead to the formation of cysteine sulfoxide and to the oxidation of tryptophane. Consequently, it is important to carefully select the  
20 excipient. pH is another key factor, and it may be necessary to buffer the product under slightly acidic conditions (pH 5 to 6).

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the  
25 methods well known in the art of pharmacy. All methods include the step of bringing the active ingredient(s) into association with the carrier which constitutes one or more accessory ingredients.

In general, the formulations for tablets or  
30 powders are prepared by uniformly and intimately blending



the active ingredient with finely divided solid carriers, and then, if necessary, as in the case of tablets, forming the product into the desired shape and size.

Formulations suitable for parenteral (e.g., intravenous) administration, on the other hand, conveniently comprise sterile aqueous solutions of the active ingredient(s). Preferably, the solutions are isotonic with the blood of the subject to be treated. Such formulations may be conveniently prepared by dissolving solid active ingredient(s) in water to produce an aqueous solution, and rendering said solution sterile.

The formulation may be presented in unit or multi-dose containers, for example, sealed ampoules or vials.

Formulations suitable for sustained release parenteral administrations (e.g., biodegradable polymer formulations such as polyesters containing lactic or glycolic acid residues) are also well known in the art. See, e.g., U.S. Patent Nos. 3,773,919 and 4,767,628 and PCT Publication No. WO 94/15587.

The somatostatin or somatostatin agonist may also be administered with another compound capable of lowering blood levels of triglycerides, cholesterol, or glycerol, such as fibrates (e.g., bezafibrate, gemfibrozil, and clofibrate), HMG-CoA reductase inhibitors (e.g., pravastatin, simvastatin, and fluvastatin, Atorvastatin, and Lovastatin), bile acid binding resins (e.g., cholestyramine and colestipol), nicotinic acid compounds (e.g., nicotinic acid and niceritrol), and fish oils. See Workshop Treatment of Hyperlipidemia 1996-2 (Lakemedelsverket, Uppsala, Sweden, 1996).

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments and from the claims.

5                   DETAILED DESCRIPTION OF THE INVENTION

It is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely  
10 illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art  
15 to which this invention belongs. Also, all publications, patent applications, patents, and other references mentioned herein are incorporated by reference.

Somatostatin and its Agonists

20                   Somatostatin (somatotropin release inhibiting factor or SRIF) has both a 14 amino acid isoform (somatostatin-14) and a 28 amino acid isoform (somatostatin-28). See Wilson, J. & Foster, D., *Williams Textbook of Endocrinology*, p. 510 (7th ed., 1985). The  
25 compound is an inhibitor of secretion of the growth hormone and was originally isolated from the hypothalamus. Brazeau, et al., *Science* 179:77 (1973). Native somatostatin has a very short duration of effect *in vivo* since it is rapidly inactivated by endo- and  
30 exopeptidase. Many novel analogs (e.g., peptide and non-

peptide compounds) have been prepared in order to enhance the duration of effect, biological activity, and selectivity (e.g., for the particular somatostatin receptor) of this hormone. Such analogs of somatostatin will be called "somatostatin agonists" herein.

Various somatostatin receptors (SSTRs) have been isolated, e.g., SSTR-1, SSTR-2, SSTR-3, SSTR-4, and SSTR-5. Thus, the somatostatin agonist may be a SSTR-1 agonist, SSTR-2 agonist, SSTR-3 agonist, SSTR-4 agonist of a SSTR-5 agonist. What is meant by a somatostatin type-5 receptor agonist (i.e., SSTR-5 agonist) is a compound which (1) has a high binding affinity (e.g.,  $K_i$  of less than 5 nM or preferably less than 2 nM or less than 1 nM) for SSTR-5 (e.g., as defined by the receptor binding assay described below) and (2) decreases lipid levels (e.g., cholesterol, glycerols, or triacylglycerols) in a patient (e.g., as shown by the biological assay described below). What is meant by a somatostatin type-5 receptor selective agonist is a somatostatin agonist which (1) has a higher binding affinity (i.e.,  $K_i$ ) for SSTR-5 than for either SSTR-1, SSTR-2, SSTR-3, or SSTR-4 and (2) decreases lipid levels (e.g., cholesterol, glycerols, or triacylglycerols) in a patient (e.g., as shown by the biological assay described below). In one embodiment, the SSTR-5 selective agonist has a  $K_i$  for SSTR-5 that is at least 2 times (e.g., at least 5 times or at least 10 times) less than its  $K_i$  for the SSTR-2 receptor (e.g., as defined by the receptor binding assay described below). In one embodiment, the

somatostatin type-5 receptor selective agonist is also a SSTR-5 agonist.

Examples of somatostatin agonists are those covered by formulae or those specifically recited in the publications set forth below, all of which are hereby incorporated by reference.

EP Application No. P5 164 EU (Inventor: G. Keri);  
Van Binst, G. et al. Peptide Research 5:8 (1992);  
Horvath, A. et al. Abstract, "Conformations of  
10 Somatostatin Analogs Having Antitumor Activity", 22nd  
European peptide Symposium, September 13-19, 1992,  
Interlaken, Switzerland;

PCT Application No. WO 91/09056 (1991);  
EP Application No. 0 363 589 A2 (1990);  
15 U.S. Patent No. 4,904,642 (1990);  
U.S. Patent No. 4,871,717 (1989);  
U.S. Patent No. 4,853,371 (1989);  
U.S. Patent No. 4,725,577 (1988);  
U.S. Patent No. 4,684,620 (1987);  
20 U.S. Patent No. 4,650,787 (1987);  
U.S. Patent No. 4,603,120 (1986);  
U.S. Patent No. 4,585,755 (1986);  
EP Application No. 0 203 031 A2 (1986);  
U.S. Patent No. 4,522,813 (1985);  
25 U.S. Patent No. 4,486,415 (1984);  
U.S. Patent No. 4,485,101 (1984);  
U.S. Patent No. 4,435,385 (1984);  
U.S. Patent No. 4,395,403 (1983);  
U.S. Patent No. 4,369,179 (1983);  
30 U.S. Patent No. 4,360,516 (1982);

- U.S. Patent No. 4,358,439 (1982);  
U.S. Patent No. 4,328,214 (1982);  
U.S. Patent No. 4,316,890 (1982);  
U.S. Patent No. 4,310,518 (1982);  
5 U.S. Patent No. 4,291,022 (1981);  
U.S. Patent No. 4,238,481 (1980);  
U.S. Patent No. 4,235,886 (1980);  
U.S. Patent No. 4,224,199 (1980);  
U.S. Patent No. 4,211,693 (1980);  
10 U.S. Patent No. 4,190,648 (1980);  
U.S. Patent No. 4,146,612 (1979);  
U.S. Patent No. 4,133,782 (1979);  
U.S. Patent No. 5,506,339 (1996);  
U.S. Patent No. 4,261,885 (1981);  
15 U.S. Patent No. 4,728,638 (1988);  
U.S. Patent No. 4,282,143 (1981);  
U.S. Patent No. 4,215,039 (1980);  
U.S. Patent No. 4,209,426 (1980);  
U.S. Patent No. 4,190,575 (1980);  
20 EP Patent No. 0 389 180 (1990);  
EP Application No. 0 505 680 (1982);  
EP Application No. 0 083 305 (1982);  
EP Application No. 0 030 920 (1980);  
PCT Application No. WO 88/05052 (1988);  
25 PCT Application No. WO 90/12811 (1990);  
PCT Application No. WO 97/01579 (1997);  
PCT Application No. WO 91/18016 (1991);  
U.K. Application No. GB 2,095,261 (1981); and  
French Application No. FR 2,522,655 (1983).

Examples of SSTR-5 selective somatostatin agonists include, but are not limited to, the following somatostatin analogs which are disclosed in the above-cited references:

- 5        H-Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> (BIM-23268);  
          H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub> (BIM-23052);  
          H-Cys-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys-NH<sub>2</sub> (BIM-23284);  
          H-Cys-Phe-Tyr-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> (BIM-23295);  
          H-Cys-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> (BIM-23313);

- 10        HO(CH<sub>2</sub>)<sub>2</sub>-N            N-(CH<sub>2</sub>)-CO-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>  
          (BIM-23272); and  
          HO(CH<sub>2</sub>)<sub>2</sub>-N            N-(CH<sub>2</sub>)<sub>2</sub>-SO<sub>2</sub>-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>

Note that for all somatostatin agonists described  
 15    herein, each amino acid residue represents the structure  
      of -NH-C(R)H-CO-, in which R is the side chain (e.g., CH<sub>3</sub>  
      for Ala). Lines between amino acid residues represent  
      peptide bonds which join the amino acids. Also, where  
      the amino acid residue is optically active, it is the L-  
 20    form configuration that is intended unless D-form is  
      expressly designated. A disulfide bond (e.g., a  
      disulfide bridge) exists between the two free thiols of  
      the Cys residues; however, it is not shown.

## 25    Synthesis of somatostatin agonists

The methods for synthesizing somatostatin agonists is well documented and are within the ability of a person of ordinary skill in the art.

Synthesis of short amino acid sequences is well established in the peptide art. For example, synthesis of H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>, described above, can be achieved by following the protocol set forth in Example I of European Patent Application 0 395 417 A1. The synthesis of somatostatin agonists with a substituted N-terminus can be achieved, for example, by following the protocol set forth in WO 88/02756, European Patent Application No. 0 329 295, and PCT Publication No. WO 94/04752.

#### Somatostatin Receptor Binding Assays

The human SSTR-1, SSTR-2, SSTR-3, SSTR-4, and SSTR-5 cDNA clones have been described (SSTR-1 and SSTR-2 in Yamada, Y., et al., Proc. Natl. Acad. Sci. USA., 89:251-255 (1992); SSTR-3 in Yamada, et al., Mol. Endocrinol. 6:2136-2142 (1993); and SSTR-4 and SSTR-5 in Yamada, et al., Biochem. Biophys. Res. Commun. 195:844-852 (1993)) and are also available from American Type Culture Collection (ATCC, Rockville, MD) (ATCC Nos. 79044 (SSTR-1), 79046 (SSTR-2), and 79048 (SSTR-3)). Based on the restriction endonuclease maps, the entire coding region of each SSTR cDNA may be excised by suitable restriction endonuclease digestion (Maniatis, T., et al., *Molecular Cloning - A Laboratory Manual*, CSHL, 1982). Restriction endonucleases are available from New England Biolabs (Beverly, MA). This cDNA fragment was inserted into the mammalian expression vector, pCMV (Russell, D., et al., J. Biol. Chem., 264:8222-8229 (1989)), using standard molecular biology techniques (see e.g., Maniatis, T., et al., *Molecular Cloning, -A Laboratory*

Manual, Cold Spring Harbor Laboratory, 1982) to produce the expression plasmid, pCMV-human SSTR-1 through pCMV-human SSTR-5. Other mammalian expression vectors include pcDNA1/Amp (Invitrogen, Sandlesy, CA). The expression  
5 plasmids were introduced into the suitable bacterial host, E. Coli HB101 (Stratagene, La Jolla, CA) and plasmid DNAs, for transfection, were prepared on Cesium Chloride gradients.

CHO-K1 (ovary, Chinese hamster) cells were  
10 obtained from ATCC (ATCC No. CCL 61). The cells were grown and maintained in Ham's F12 media (Gibco BRL, Grand Island, NY) supplemented with 10% fetal bovine serum under standard tissue culture conditions. For transfection, the cells were seeded at a density  $1 \times 10^6$ /60-cm plate (Baxter Scientific Products, McGraw Park,  
15 IL.). DNA mediated transfection was carried out using the calcium phosphate co-precipitation method (Ausubel, F.M., et al., Current Protocols in Molecular Biology, John Wiley & Sons, 1987). The plasmid pRSV-neo (ATCC;  
20 ATCC No. 37198) was included as a selectable marker at 1/10 the concentration of the expression plasmid. CHO-K1 clonal cell lines that have stably inherited the transfected DNA were selected for growth in Ham's F12 media containing 10% fetal bovine serum and 0.5mg/ml of  
25 G418 (Sigma). The cells were ring-cloned and expanded in the same media for analysis.

Expression of the human SSTR-1 through SSTR-5 receptors in the CHO-K1 cells were detected by Northern blot analysis of total RNA prepared from the cells  
30 (Sambrook, J.E., et al., Molecular Cloning - A Laboratory



Manual, Ed. 2., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989) and by receptor binding using [<sup>125</sup>I-Tyr<sup>11</sup>]somatostatin-14 as a ligand. Transfected cell lines expressing the human SSTR receptors were clonally expanded in culture and used in the following SSTR binding protocol.

Crude membranes were prepared by homogenization of the transfected cells in 20 ml of ice-cold 50 mM Tris-HCl with a POLYTRON homogenizer (setting 6, 15 sec). Buffer was added to obtain a final volume of 40 ml, and the homogenate was centrifuged in a Sorval SS-34 rotor at 39,000 g for 10 min at 0-4°C. The resulting supernatant was decanted and discarded. The pellet was rehomogenized in ice-cold buffer, diluted, and centrifuged as before. The final pellet was resuspended in the 10 mM Tris HCl and held on ice for the receptor binding assay.

Aliquots of the membrane preparation were incubated for 30 min at 30°C with 0.05 nM [<sup>125</sup>I-Tyr<sup>11</sup>]somatostatin-14 (2000 Ci/mmol; Amersham Corp., Arlington Heights, IL) in 50 mM HEPES (pH 7.4) containing a test somatostatin agonist of various concentrations (e.g., 10<sup>-11</sup> to 10<sup>-6</sup>), 10 mg/ml bovine serum albumin (fraction V) (Sigma Chemical Co., St. Louis, MO), MgCl<sub>2</sub> (5 mM), Trasylol (200 KIU ml), bacitracin (0.02 mg/ml), and phenylmethanesulphonyl fluoride (0.02 mg/ml). The final assay volume was 0.3 ml. The incubations were terminated by rapid filtration through GF/C filters (pre-soaked in 0.3% polyethylenimine for 30 min) using a Brandel filtration manifold. Each tube and filter were then

washed three times with 5 ml aliquots of ice-cold buffer.

Specific binding was defined as the total [ $^{125}$ I-Tyr $^{11}$ ]SRIF-14 bound minus that bound in the presence of 1000 nM. The  $K_i$  values for the tested somatostatin

5 agonists were calculated by using the following formula:

$K_i = IC_{50} / [1 + (LC/LEC)]$  where  $IC_{50}$  is the concentration of test somatostatin agonist required to inhibit 50 percent of the specific binding of the radioligand [ $^{125}$ I-Tyr $^{11}$ ]somatostatin-14, LC is the concentration of the

10 radioligand (0.05 nM), and LEC is the equilibrium dissociation constant of the radioligand (0.16 nM). The  $K_i$  values (nM) for the tested somatostatin agonists are shown in Table I.

TABLE I

	hSSTR-1	hSSTR-2	hSSTR-3	hSSTR-4	hSSTR-5
Somatostatin-14	2.26	0.23	1.2	1.8	1.41
Somatostatin-28	2.38	0.30	1.3	7.93	0.4
BIM-23268	1227	15.06	545	3551	0.42
BIM-23052	97.6	11.96	5.6	127	1.22
BIM-23272	47.7	3.23	10.9	753	1.01
BIM-23284	27.9	19.3	35.6	58.6	0.85
BIM-23295	86.9	6.19	9.7	3.4	0.34
BIM-23313	151	4.78	25.5	55.3	0.30

15

#### Reduction of Glycerol and Triglycerides

The obese (fa/fa) Zucker and its derivative in the Zucker diabetic fatty (ZDF/Drt-fa) are excellent models of diabetes-induced dyslipidemia (Shafrir, E.,

20 Diabetes/Metabolism Rev. 8:179-208 (1992); Peterson,

R.G., et al., ILAR News 32:16-19 (1990)). The animals develop progressive hypertriglyceridemia and hypercholesterolemia.

The effect of chronic treatment with BIM-23268 on plasma lipids was examined in an obese animal model, the fatty (fa/fa) Zucker rats (Bray, G., Federation Proceedings 36:q48-153 (1977)) (purchased from Harlan-Olac, Bicester, Oxon, U.K.). Eleven male fatty Zucker rats weighing about 450 grams were randomly divided into 2 groups and their initial body weights recorded. The animals were housed in pairs in a normal 12 hour light/dark cycle at 20 " 21C and fed a standard laboratory rat diet (Beekay rat and mouse diet, Bantin & Kingman, Hull, Humberside, U.K.) overnight *ad libitum*.

For the group assigned to receive drug treatment, the rats received BIM-23268C at 3 mg/kg, by subcutaneous injection twice a day at 10:00 a.m. and 5:00 p.m. The other group was treated with a subcutaneous injection of 0.1 ml/100 g of saline twice a day at 10:00 a.m. and 5:00 p.m. The animals were subjected to the BIM-23268 or saline treatment for a total of six days.

On the last day of treatment (day 6), food was removed at 5:00 p.m. and the rats fasted overnight. At 9:00 a.m. the next day, the animals were subjected to a glucose challenge, given as a 0.8 gram/kg of glucose orally. Periodic 400 ul of blood samples were taken from the tail vein (Peterson, R.G., ILAR News 32:16-19 (1990)) at 60 min. and 30 min. before, and at 30, 60, 90, and 120 min. after the administration of the glucose challenge (0.8 gram/kg orally). Aprotinin (Traysylol, Bayer UK,

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Hayward's Heath, W. Sussex, U.K.) and heparin (Sigma Chemical Co., Poole, Dorset, U.K.) were added to the blood samples to a final concentration of 400 KIU/ml and 100 units/ml, respectively. Plasma fractions were  
5 prepared from these samples by centrifugation at 400 x G in a microfuge, for the estimation of triglycerides and glycerol. Samples were then stored at -80°C until assayed.

Plasma glycerol and triglycerides were determined  
10 using the Sigma Enzymatic (Tinder) calorimetric assay kit (Cat #337-B, Sigma Chemical Co., Poole, Dorset, U.K.) and measuring absorbance at 540 nm in a spectrophotometer.

After 6 days of treatment with BIM-23268C at 3 mg/kg, twice a day by subcutaneous injection, both plasma  
15 glycerol and triglycerides were significantly lowered, as exemplified by the samples taken at time 30 and 60 min. before the oral glucose challenge. See Fig. 1 and Fig. 2. The administration of an oral glucose challenge had no significant effect on plasma lipids. The BIM-23268C  
20 treated group showed significantly lower plasma glycerol and triglycerides through the 2-hour test period. The results suggested that BIM-23268C, following a 6-day treatment period at the prescribed dose was effective in reducing hypertriglyceridemia.

25

#### Measure of Efficacy in Patient

The effect of the somatostatin agonist will be assessed for a reduction in total cholesterol, total triglycerides, and total LDL cholesterol (e.g., as  
30 described in Dubrey, S.W., et al., Diabetes 43:831-835

(1994). The long term effect of the drug is examined by the change in coronary artery disease (Reviewed in Donahue, The Endocrinologist, 4:112-116 (1994)).

5

#### OTHER EMBODIMENTS

The foregoing description has been limited to specific embodiments of this invention. It will be apparent, however, that variations and modifications may be made to the invention, with the attainment of some or  
10 all of the advantages of the invention. Such embodiments are also within the scope of the following claims.

CLAIMS

1. A method of treating hyperlipidemia in a patient, said method comprising administering a  
5 therapeutically effective amount of a somatostatin type-5 receptor agonist to said patient.

2. A method of claim 1, wherein said somatostatin type-5 receptor agonist has a  $K_i$  of less than 2 nM for the somatostatin type-5 receptor.

10 3. A method of treating hyperlipidemia in a patient, said method comprising administering a therapeutically effective amount of a somatostatin type-5 receptor selective agonist to said patient.

4. A method of claim 3, wherein said  
15 somatostatin type-5 receptor selective agonist has a  $K_i$  for the type-5 somatostatin receptor that is at least 10 times less than its  $K_i$  for the somatostatin type-2 receptor.

5. A method of claim 1, said method comprising  
20 administering a therapeutically effective amount of H-Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub>, where a disulfide bond exists between the free thiols of the two Cys residues, or H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>.

6. A method of lowering the amount of  
25 triacylglycerols, glycerol, or cholesterol in the blood of a patient, said method comprising administering a therapeutically effective amount of a somatostatin type-5 receptor agonist to said patient.

7. A method of lowering the amount of  
30 triacylglycerols, glycerol, or cholesterol in the blood

of a patient, said method comprising administering a therapeutically effective amount of a somatostatin type-5 receptor selective agonist to said patient.

8. A method of claim 6, wherein said method  
5 comprises lowering the amount of triacylglycerols in said patient.

9. A method of claim 8, wherein said somatostatin type-5 receptor agonist has a  $K_i$  of less than 2 nM for the somatostatin type-5 receptor.

10 10. A method of claim 7, wherein said method comprises lowering the amount of triacylglycerols in said patient.

11. A method of claim 10, wherein said somatostatin type-5 receptor selective agonist has a  $K_i$   
15 for the type-5 somatostatin receptor that is at least 10 times less than its  $K_i$  for the somatostatin type-2 receptor.

12. A method of claim 8, said method comprising  
administering a therapeutically effective amount of H-  
20 Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub>, where a disulfide bond exists between the free thiols of the two Cys residues, or H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>.

13. A method of claim 6, wherein said method  
comprises lowering the amount of glycerol in said  
25 patient.

14. A method of claim 13, wherein said somatostatin type-5 receptor agonist has a  $K_i$  of less than 2 nM for the somatostatin type-5 receptor.

15. A method of claim 7, wherein said method comprises lowering the amount of glycerol in said patient.

16. A method of claim 15, wherein said  
5 somatostatin type-5 receptor selective agonist has a  $K_i$  for the type-5 somatostatin receptor that is at least 10 times less than its  $K_i$  for the somatostatin type-2 receptor.

17. A method of claim 13, said method comprising  
10 administering a therapeutically effective amount of H-Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub>, where a disulfide bond exists between the free thiols of the two Cys residues, or H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>.

18. A method of claim 6, wherein said method  
15 comprises lowering the amount of cholesterol in said patient.

19. A method of claim 18, wherein said somatostatin type-5 receptor agonist has a  $K_i$  of less than 2 nM for the somatostatin type-5 receptor.

20. A method of claim 7, wherein said method  
20 comprises lowering the amount of total cholesterol or LDL cholesterol in said patient.

21. A method of claim 20, wherein said somatostatin type-5 receptor selective agonist has a  $K_i$   
25 for the type-5 somatostatin receptor that is at least 10 times less than its  $K_i$  for the somatostatin type-2 receptor.

22. A method of claim 18, said method comprising administering a therapeutically effective amount of H-  
30 Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub>, where a disulfide



bond exists between the free thiols of the two Cys residues, or H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>.

23. A method according to claim 1 wherein the somatostatin type-5 receptor agonist is
- 5 H-Cys-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys-NH<sub>2</sub> ,  
 H-Cys-Phe-Tyr-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> ,  
 H-Cys-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> ,

HO(CH<sub>2</sub>)<sub>2</sub>-N N-(CH<sub>2</sub>)<sub>2</sub>-CO-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>

or

HO(CH<sub>2</sub>)<sub>2</sub>-N N-(CH<sub>2</sub>)<sub>2</sub>-SO<sub>2</sub>-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>

10

24. A method according to claim 8 wherein the somatostatin type-5 receptor agonist is
- H-Cys-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys-NH<sub>2</sub> ,
- 15 H-Cys-Phe-Tyr-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> ,  
 H-Cys-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> ,

HO(CH<sub>2</sub>)<sub>2</sub>-N N-(CH<sub>2</sub>)<sub>2</sub>-CO-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>

or

HO(CH<sub>2</sub>)<sub>2</sub>-N N-(CH<sub>2</sub>)<sub>2</sub>-SO<sub>2</sub>-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>

20

25. A method according to claim 13 wherein the somatostatin type-5 receptor agonist is
- H-Cys-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys-NH<sub>2</sub> ,  
 H-Cys-Phe-Tyr-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> ,

H-Cys-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> ,

HO(CH<sub>2</sub>)<sub>2</sub>-N — N-(CH<sub>2</sub>)<sub>2</sub>-CO-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>

or

HO(CH<sub>2</sub>)<sub>2</sub>-N — N-(CH<sub>2</sub>)<sub>2</sub>-SO<sub>2</sub>-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>

5 .

26. A method according to claim 18 wherein the somatostatin type-5 receptor agonist is

H-Cys-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys-NH<sub>2</sub> ,

10 H-Cys-Phe-Tyr-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> ,

H-Cys-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys-NH<sub>2</sub> ,

HO(CH<sub>2</sub>)<sub>2</sub>-N — N-(CH<sub>2</sub>)<sub>2</sub>-CO-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>

or

HO(CH<sub>2</sub>)<sub>2</sub>-N — N-(CH<sub>2</sub>)<sub>2</sub>-SO<sub>2</sub>-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>

15 .

27. A pharmaceutical composition comprising a therapeutically effective amount of a somatostatin type-5 receptor, optionally selective, agonist.

20 28. A pharmaceutical composition as claimed in claim 27, said agonist having the features identified in any one of claims 2, 4, 5 and 23 to 26.

29. Use of a somatostatin type-5 receptor, optionally selective, agonist in the formulation of a  
25 pharmaceutical composition for use in treating hyperlipidemia, or reducing the amount of

25

tracylglycerols, glycerol, or cholesterol in a human or mammalian animal.

30. Use of a somatostatin agonist according to claim 29, wherein said somatostatin agonist has the  
5 relevant features identified in any one of claims 2, 4, 5 and 23 to 26.

31. A pharmaceutical composition substantially as hereinbefore described with reference to the Examples.

10

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 98/02998

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K38/31

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 35950 A (UNIVERSITY OF BUCKINGHAM) 14 November 1996 see page 5, line 1 - line 3; claims 10-15 see page 8, line 24 - page 9, line 10 ---	27, 28, 31
X	WO 97 11962 A (BIOMEASURE INCORPORATED ET AL.) 3 April 1997 see page 6, line 12 - line 23; claim 21 see page 12, line 22 - page 13, line 35; table 1 --- -/--	27, 28, 31



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

### \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

22 September 1998

Date of mailing of the international search report

07/10/1998

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/02998

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DAVENPORT M ET AL: "SOMATOSTATIN TYPE 5 RECEPTORS MEDIATE THE INHIBITORY ACTION OF SOMATOSTATIN ON INSULIN SECRETION IN ZUCKER FATTY RAT PERFUSED PANCREAS" DIABETOLOGIA, vol. 38, no. SUPPL. 01, 1995, page A106 XP000602793 see abstract nr. 410 ----	1-31
A	N. MOLLER ET AL.: "EFFECTS OF THE SOMATOSTATIN ANALOGUE SMS 201-995 (SANDOSTATIN) ON MOUTH-TO-CAECUM TRANSIT TIME AND ABSORPTION OF FAT AND CARBOHYDRATES IN NORMAL MAN." CLINICAL SCIENCE, vol. 75, no. 4, October 1988, pages 345-350, XP002078328 LONDON, GB cited in the application see the whole document ----	1-31
P,X	WO 98 10786 A (Y. COHEN) 19 March 1998  see page 1, line 29 - line 31: claims 1,19.58 see page 3, line 13 - page 4, line 13 see page 23, line 28 - page 24, line 5 -----	1-22, 27-31

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 98/02998

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
**Remark:** Although claims 1-26  
are directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/EP 98/02998

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9635950 A	14-11-1996	US 5763200 A	09-06-1998
		AU 694360 B	16-07-1998
		AU 5764596 A	29-11-1996
		CA 2220106 A	14-11-1996
		EP 0829011 A	18-03-1998
WO 9711962 A	03-04-1997	US 5708135 A	13-01-1998
		AU 6914596 A	17-04-1997
		EP 0859785 A	26-08-1998
		NO 981395 A	27-03-1998
WO 9810786 A	19-03-1998	AU 4133997 A	02-04-1998

